PATENT ABSTRACTS OF JAPAN

(11)Publication number : 2002-335949
(43)Date of publication of application : 26.11.2002

(51)Int.Cl. C12N 1/00
B32B 3/12
C08J 9/28
C08L 67/00
C12M 1/00
//(C08L 67/00
C08L101:00)

(21)Application number : 2001-152379
(22)Date of filing : 22.05.2001

(71)Applicant : INST OF PHYSICAL & CHEMICAL RES
(72)Inventor : SHIMOMURA MASATSUGU
NISHIKAWA TAKEHIRO
ARAI KEIKO

(54) CELL THREE-DIMENSIONAL TISSUE CULTURE METHOD USING HONEYCOMB STRUCTURE FILM

(57)Abstract:
PROBLEM TO BE SOLVED: To develop a culture method for forming an ordered cell three-dimensional aggregate similar to a biological tissue.
SOLUTION: This culture method for forming the cell three-dimensional aggregate is characterized by casting the hydrophobic organic solvent solution of a biodegradable and amphipathic polymer alone or a polymer mixture comprising a biodegradable polymer and an amphipathic polymer on a substrate, evaporating the organic solvent and simultaneously condensing dew on the surface of the cast liquid, evaporating fine water drops formed by the condensation of the dew to form a honeycomb structure film, and then culturing cells on the honeycomb structure film or its oriented film as a substrate for culturing the cells.

LEGAL STATUS

[Date of request for examination]

[Date of sending the examiner's decision of rejection]

[Kind of final disposal of application other than the examiner's decision of rejection or application converted registration]

[Date of final disposal for application]

[Patent number]

[Date of registration]

[Number of appeal against examiner's decision of rejection]

[Date of requesting appeal against examiner's decision of rejection]

[Date of extinction of right]

* NOTICES *

1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

CLAIMS

[Claim(s)]
[Claim 1] The cast of the hydrophobic organic solvent solution of the polymer mixture which consists of the independent polymer or biodegradability polymer which has biodegradability and amphiphilic, and an amphiphilic polymer is carried out on a substrate. It is made to dew on this cast liquid front face at the same time it transpires this organic solvent, and it is characterized by cultivating a cell, using the honeycomb structure object film obtained by evaporating the minute waterdrop produced by this dew condensation, or its oriented film as a base material for cell cultures. How to form the three-dimensions aggregate of a cell.
[Claim 2] The approach according to claim 1 of using aliphatic series polyester as a biodegradability polymer.
[Claim 3] The approach according to claim 1 or 2 of using the polymer mixture which consists of 50 - 99 w/w% of biodegradability polymer, and 50 - 1 w/w% of amphiphilic polymer as polymer mixture which consists of a biodegradability polymer and an amphiphilic polymer.
[Claim 4] The approach given in any of claims 1-3 they are using the honeycomb structure object film obtained by evaporating the minute waterdrop which carried out the cast of the hydrophobic organic solvent solution on the substrate, was made to dew on this cast liquid front face while transpiring this organic solvent by spraying high humidity air, and was produced by this dew condensation, or its oriented film.
[Claim 5] The approach given in any of claims 1-3 they are using the honeycomb structure object film obtained by evaporating the minute waterdrop which carried out the cast of the hydrophobic organic solvent solution on the substrate under atmospheric air of 50 - 95% of relative humidity, was made to dew on this cast liquid front face while transpiring this organic solvent, and was produced by this dew condensation, or its oriented film.
[Claim 6] The approach given in any of claims 1-5 they are using the oriented film of a honeycomb structure object film.
[Claim 7] An approach given in any of claims 1-6 which extend by uniaxial stretching, biaxial stretching, or triaxial extension they are.
[Claim 8] The approach given in any of claims 1-7 they are the rate of expanding of the extension direction is within the limits of 1.1 to 10 times.
[Claim 9] The approach given in any of claims 1-8 they are the diameter of a honeycomb structure object is 0.1-100 micrometers.
[Claim 10] An approach given in any of claims 1-9 which are characterized by cultivating a cell on both sides of a honeycomb structure object film or its oriented film they are.
[Claim 11] An approach given in any of claims 1-10 which are characterized by the cells cultivated on both sides of a honeycomb structure object film or its oriented film differing mutually they are.
[Claim 12] The three-dimensions aggregate of a cell prepared by the approach given in any of claims 1-11 they are.

[Translation done.]

* NOTICES *

1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] This invention relates to the three-dimensions tissue culture method of a cell for having used the honeycomb structure object film. This invention relates to the approach of forming the three-dimensions aggregate of a cell characterized by cultivating a cell using the honeycomb structure object film prepared using a biodegradability polymer and an amphiphilic polymer, or its oriented film more at a detail.
[0002]
[Description of the Prior Art] In the interaction of a cell and an ingredient, it is known that a cell will be influenced not only with the chemical property on the front face of an ingredient but with a detailed configuration. Then, when aiming at functional control of a cell from viewpoints, such as systems engineering, processing of the chemical property on the front face of an ingredient in contact with a cell and the both sides of detailed structure becomes important. Installation of the size control of the cell adhesion side which used the micro pattern technique used for semiconductor industry etc. as a surface treatment technique as a surface micro-processing method, and the minute slot structure to a substratum, production of the detailed irregularity by the microsphere, etc. are mentioned, and it is known that the surface fine structure will affect growth of a cell etc. greatly.
[0003] A very advanced technique is required for a surface setup using these micro pattern techniques, and the present condition is having many problems, such as mass production method being impossible and becoming high cost. It is known that the film which has the honeycomb structure of mum scale by carrying out the cast of the dilute solution of the polymer which has structure completely special as another surface patterning technique under high humidity will be obtained. It is the description that this approach is excellent in the economical efficiency which hits to carry out patterning.
[0004] the rod-coil which will specifically become 283 volumes and a page 373 from a hydrophilic block and a hydrophobic block Science and 1999 -- a jib -- the example using the polyphenyl quinoline-block-polystyrene which is a lock polymer -- moreover, the jib which will become 369 volumes and a page 387 from polystyrene and the poly para-phenylene which is an upright block Nature and 1994 -- the example using a lock polymer is indicated. Thus, at the Prior art, the special polymer having a part with the strong autoagglutination force and the part which discovers flexibility was used, these polymers were dissolved in the hydrophobic organic solvent, and the honeycomb structure object was prepared by carrying out the cast of this. On the other hand, this invention persons are SHINSO lids. Films, 1998, 327 to 329 volumes, A page 854, Supra molecular Science, 1998, the 5th volume, A page 331 and molecular crystal liquid crystal, The acrylamide polymer of a hydrophilic property will be made into a principal chain frame in 1998 at the 322nd volume and a page 305. The amphiphilic polymer which has a lactose radical or a carboxyl group as the dodecyl and a hydrophilic side chain as a hydrophobic side chain, Or it has reported giving the thin film which has honeycomb structure by the approach with the same ion complex of anionic polysaccharides, such as heparin and dextran sulfate, and the long-chain alkyl ammonium salt of the 4th class.

[0005] However, since it was inferior to the self-independence nature of the honeycomb structure object acquired in these polymers or had the fault of honeycomb structure collapsing with time, it was not what offers function sufficient as a base material for cell cultures.
[0006] When performing a cell culture in a cell technology, systems engineering, etc., the base material used as the scaffold of a cell is required, and it is known in the interaction with a cell like the above-mentioned that a cell will be influenced not only with the chemical property on the front face of best but with a detailed configuration. When aiming at functional control of a cell, a design of the chemical property on the front face of an ingredient and the both sides of structure with a detailed cell in contact with a cell becomes important. With the porous film which has honeycomb structure, a honeycomb pattern offers a cell adhesion side and it is shown that a vesicular structure serves as access to the support base of a cell and the supply root of a nutrition.
[0007] If a cell is systematized based on this honeycomb structure film, an artificial organ can be considered as that one usage. However, since embedding inside of the body becomes indispensable when it is made an artificial organ etc., as for this base material, being absorbed to the living body is desirable in the long run. The time amount which a cell culture takes with the ingredient which gives old honeycomb structure maintains structure to stability, and there is nothing that was made from a biodegradability ingredient which is disassembled more than at it. In other words, in combining a honeycomb structure object, a cell technology, and a cell culture technique, and developing to medical-application ways, such as an artificial organ, it is indispensable to use a biodegradability ingredient.
[0008] In view of such a situation, as for this invention persons, a biodegradability polymer the hydrophobic organic solvent solution of a polymer with which 50 - 99 w/w% and an amphiphilic polymer consist of 50 - 1 w/w% It is made to dew on this cast liquid front face at the same time it carries out the cast on a substrate under atmospheric air of 50 - 95% of relative humidity and transpires this organic solvent gradually. The honeycomb structure object acquired by evaporating the minute waterdrop produced by this dew condensation and the film which becomes a list from this honeycomb structure object are proposed (Japanese-Patent-Application-No. No. 340568 [11 to] specification (it sets at this time and is un-opening to the public).). However, it was unknown whether the three-dimensions aggregate of the cell which was order similar to a body tissue could be formed using the film which has the honeycomb structure produced by this approach.
[0009]
[Problem(s) to be Solved by the Invention] This invention made it the technical problem which should be solved to develop the cultivation for forming the three-dimensions aggregate of the cell which was order similar to a body tissue out of a living body.
[0010]
[Means for Solving the Problem] this invention persons found out that the three-dimensions-cell aggregation object structure of liver tissue resemblance could be made to form by cultivating a hepatocyte on the free-standing honeycomb structure object of polylactic acid, as a result of inquiring wholeheartedly, in order to solve the above-mentioned technical problem. Moreover, when the cardiac muscle cell was cultivated on the free-standing honeycomb structure object of polylactic acid, it found out that the three-dimensions myocardium cell aggregation object which the cardiac muscle cell pasted up on each side of a film pasted up through the vesicular structure was formed. This invention is completed based on these knowledge.
[0011] Namely, according to this invention, the cast of the hydrophobic organic solvent solution of the polymer mixture which consists of the independent polymer or biodegradability polymer which has biodegradability and amphiphilic, and an amphiphilic polymer is carried out on a substrate. It is made to dew on this cast liquid front face at the same time it transpires this organic solvent, and it is characterized by cultivating a cell, using the honeycomb structure object film obtained by evaporating the minute waterdrop produced by this dew condensation, or its oriented film as a base material for cell cultures. The approach of forming the three-dimensions aggregate of a cell is offered.
[0012] In this invention, the hydrophobic organic solvent solution of the polymer mixture which

consists of a biodegradability polymer and an amphiphilic polymer is used preferably. In this invention, aliphatic series polyester is preferably used as a biodegradability polymer. In this invention, the polymer mixture which consists of 50 - 99 w/w% of biodegradability polymer and 50 - 1 w/w% of amphiphilic polymer is preferably used as polymer mixture which consists of a biodegradability polymer and an amphiphilic polymer.

[0013] For example, carry out the cast of the hydrophobic organic solvent solution on a substrate, and it is made to dew on this cast liquid front face in this invention at the same time it transpires this organic solvent by spraying high humidity air. The honeycomb structure object film obtained by evaporating the minute waterdrop produced by this dew condensation or its oriented film can be used. Or the cast of the hydrophobic organic solvent solution is carried out on a substrate under atmospheric air of 50 - 95% of relative humidity. While transpiring this organic solvent, it can be made to be able to dew on this cast liquid front face, and the honeycomb structure object film obtained by evaporating the minute waterdrop produced by this dew condensation or its oriented film can be used.

[0014] In this invention, the oriented film of a honeycomb structure object film may be used, uniaxial stretching, biaxial stretching, or triaxial extension can perform extension in that case, and the rate of expanding of the extension direction is within the limits of 1.1 to 10 times preferably. In this invention, the diameter of a honeycomb structure object is 0.1-100 micrometers preferably. In this invention, a cell is preferably cultivated on both sides of a honeycomb structure object film or its oriented film. In this case, the cells cultivated on both sides of a honeycomb structure object film or its oriented film may differ mutually. According to another side face of this invention, the three-dimensions aggregate of a cell prepared by the approach by above-mentioned this invention is offered.

[0015]

[Embodiment of the Invention] Hereafter, the embodiment and practice of this invention are explained to a detail. The approach of forming the three-dimensions aggregate of the cell by this invention The cast of the hydrophobic organic solvent solution of the polymer mixture which consists of the independent polymer or biodegradability polymer which has biodegradability and amphiphilic, and an amphiphilic polymer is carried out on a substrate. It is made to dew on this cast liquid front face at the same time it transpires this organic solvent, and it is characterized by cultivating a cell, using the honeycomb structure object film obtained by evaporating the minute waterdrop produced by this dew condensation, or its oriented film as a base material for cell cultures.

[0016] In this invention, the mixture of two or more polymers which consist of the polymer which may use the independent polymer which has biodegradability and amphiphilic, or has biodegradability, and the polymer which has amphiphilic may be used.

[0017] As a biodegradability polymer which can be used by this invention, aliphatic series polycarbonates, such as polybutylene carbonate and polyethylene carbonate, etc. are desirable from a soluble viewpoint to an organic solvent in biodegradability aliphatic series polyester, such as polylactic acid, polyhydroxy butanoic acid, the poly caprolactone, a polyethylene horse mackerel peat, and a polybutylene horse mackerel peat, and a list. Especially, polylactic acid and the poly caprolactone are desirable from viewpoints, such as an ease of acquisition, and a price.

[0018] As an amphiphilic polymer which can be used by this invention It is desirable that there is no toxicity if it takes into consideration to use as a cell culture base material. A polyethylene glycol / polypropylene-glycol block copolymer, The amphiphilic polymer which makes an acrylamide polymer a principal chain frame and has a lactose radical or a carboxyl group as the dodecyl and a hydrophilic side chain as a hydrophobic side chain, Or the ion complex of anionic macromolecules, such as heparin, and dextran sulfate, a nucleic acid (DNA and RNA), and long-chain alkyl ammonium salt, It is desirable to use the amphiphilic polymer which made the hydrophilic radical water-soluble protein, such as gelatin, a collagen, and albumin.

[0019] Moreover, as an independent polymer which has biodegradability and amphiphilic, a polylactic acid-polyethylene-glycol block copolymer, a Pori epsilon-caprolactone-polyethylene-glycol block copolymer, a Pori malic-acid-Pori malic-acid alkyl ester block copolymer, etc. are mentioned, for example.

[0020] In creating the honeycomb structure object used by this invention, it is required to be nonaqueous solubility (hydrophobicity) as an organic solvent used from it being required to make a minute waterdrop particle form on a polymer solution. As an example of a hydrophobic organic solvent, nonaqueous solubility ketones, such as ester, such as aromatic hydrocarbon, such as halogen system organic solvents, such as chloroform and a methylene chloride, benzene, toluene, and a xylene, ethyl acetate, and butyl acetate, and methyl isobutyl ketone, a carbon disulfide, etc. are mentioned. These organic solvents may be used as a mixed solvent which combined these solvents, even if it uses it independently. The polymer concentration of the sum total of both biodegradability polymer and amphiphilic polymer which dissolves in a hydrophobic organic solvent is 0.01 to 10 % of the weight preferably, and is 0.05 to 5 % of the weight more preferably. The dynamics reinforcement of the film which will be obtained if polymer concentration is lower than 0.01 % of the weight runs short and is not desirable. Moreover, at 10 % of the weight or more, polymer concentration becomes [polymer concentration] high too much, and sufficient honeycomb structure is not acquired.

[0021] Moreover, although especially the presentation ratio is not limited when using a biodegradability polymer and an amphiphilic polymer, it is within the limits of 99:1-50:50 (wt/wt) preferably. When an amphiphilic polymer ratio is one or less, the stability of the honeycomb structure object with which it may become difficult with which for uniform honeycomb structure to obtain, and an amphiphilic polymer ratio is obtained or more by 50, and dynamic stability may fall especially.

[0022] In this invention, the cast of this polymer organic solvent solution is carried out on a substrate, and a honeycomb structure object is prepared. Liquids, such as a macromolecule which was excellent in organic solvent-proof nature, such as inorganic materials, such as glass, a metal, and a silicon wafer, polypropylene, polyethylene, and a polyether ketone, as a substrate, water, a liquid paraffin, and a liquefied polyether, can be used. Especially, when water is used for a base material, this structure can be independently taken out from a substrate easily by employing efficiently the independence nature which is the description of this honeycomb structure object, and it is suitable.

[0023] The device in which honeycomb structure is formed by this invention is considered as follows. When a hydrophobic organic solvent evaporates, in order to take the latent heat, the temperature of a cast philharmonic front face falls, and the drop of minute water condenses and adheres to a polymer solution front face. The surface tension between water and a hydrophobic organic solvent decreases, for this reason, it faces that a water particle tends to condense and it is going to become one lump, and work of the hydrophilic part in a polymer solution is stable. A solvent follows on evaporating, and it stands in a line in the form in which the drop which carried out the hexagonal form carried out the closest packing, and finally, water flies and it remains as a form where the polymer was regularly located in a line in the shape of a honeycomb.

[0024] therefore, as an environment where this film is prepared (1) Carry out the cast of the hydrophobic organic solvent solution on a substrate, and it is made to dew on this cast liquid front face at the same time it transpires this organic solvent gradually by spraying high humidity air. In a list, How to evaporate the minute waterdrop produced by this dew condensation; (2) hydrophobic organic solvent solution Approach; which evaporates the minute waterdrop which carried out the cast on the substrate under atmospheric air of 50 - 95% of relative humidity, was made to dew on this cast liquid front face while transpiring this organic solvent, and was produced by this dew condensation is desirable. Thus, although not limited, especially the magnitude of each of the made honeycomb structure objects (each) is 0.1 to 100 micrometers preferably, is 0.1 to 10 micrometers more preferably, and if it is the magnitude of this range, it can be suitably used as a base material for cell cultures.

[0025] Furthermore, in addition to using the honeycomb structure object film produced as mentioned above as it is, in this invention, the film which has the array structure of the pore elongated by extending this honeycomb structure object film can also be used. When a cell is cultivated on such an oriented film, a cell carries out orientation in accordance with the linear array of pore.

[0026] Especially the approach of extension of a film cannot be limited, for example, can pinch

two or more edges of a honeycomb structure object film by the pincette or the hand, and can perform them by pulling in the expanding direction. Or it can also extend using a micro manipulator.

[0027] In this invention, any of uniaxial stretching, biaxial stretching, or triaxial extension are sufficient as extension. The mimetic diagram of the example of the extension in this invention is shown in drawing 1 . In drawing 1 , uniaxial stretching and (b) show biaxial stretching, (c) shows triaxial extension, (a) shows the angle on which a symmetry axis and the extension direction make alpha, and beta and gamma show the angle which the extension direction makes. In this invention, although especially the rate of expanding of the extension direction is not limited, it is within the limits of 1.1 to 10 times preferably. At 1.1 or less times, the effectiveness of this invention according [the rate of expanding] to extension is small, and the rate of expanding becomes [a film] is easy to be destroyed by 10 or more times.

[0028] In this invention, the three-dimensions aggregate of a cell can be formed by cultivating a cell, using the honeycomb structure object film produced as mentioned above or its oriented film as a base material for cell cultures. Especially the class of cell which can be cultivated by the approach of this invention is not limited, but can cultivate the cultured cell of arbitration, the cell extracted from the organization in the list. The pulsation rhythm can be synchronized with the whole film, and the aggregate of the rat embryo heart origin cardiac muscle cell which the aggregate of the rat liver origin hepatocyte formed by the approach of this invention could discover high albumin composition ability, and was formed by the approach of this invention can carry out pulsation as shown by the following examples. Therefore, according to the approach of this invention, it is possible to form the three-dimension organism which discovers a function in the living body from a cultured cell.

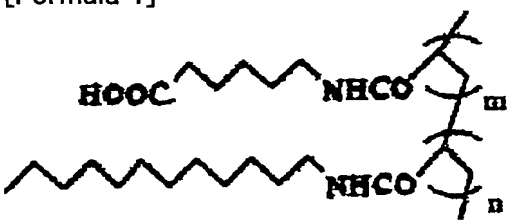
[0029] In the desirable mode of this invention, after carrying out seeding of the cell to one side of a honeycomb structure object film and checking adhesion of a cell on a film first, it cultivates by carrying out seeding of the cell to the field of the opposite side. Culture can be performed according to the conditions of the usual cell culture. That is, it is desirable to cultivate a cell in this invention on both sides of a honeycomb structure object film or its oriented film. In this case, even if the cell cultivated on both sides of a honeycomb structure object film or its oriented film is mutually the same, they may differ. Especially the class of culture medium used for a cell culture is not limited, but can choose suitable culture media (for example, culture medium which added fetal calf serum etc. to a Williams'E culture medium, F-10 culture medium, RPM11640 culture medium, the MEM culture medium of Eagle, DMEM culture media, or these culture media) according to the class of cell. a culture condition -- the class of cell -- responding -- suitably -- it can choose -- general -- pH 6-8, the temperature of 30-40 degrees C, and 5%CO -- it can cultivate under lower conditions 2 ****.

[0030] Moreover, when using an oriented film as a base material for cell cultures, it is also possible to control the array of a cell and, in a rebirth of the organization which has the array structure of a cell like the cardiac muscle tissue or a blood vessel organization especially, it is advantageous. The three-dimensions aggregate of a cell prepared by the approach by this invention which was described above is also within the limits of this invention. Hereafter, although this invention is explained to a detail using an example, this invention is not limited at all by the example.

[0031]

[Example] Example 1: 1:2.5:6.5 mixed comparatively (volume ratio) the chloroform solution (10g/L) of the production polylactic acid (molecular weight: 85,000-160,000) of a free-standing honeycomb film, the benzene solution (0.4g/L) of the amphiphilic giant molecule Cap (the chemical structure is shown below), and benzene, and the cast solution was prepared. Milli-Q water was filled in the glass petri dish with a diameter of 9cm, and it changed into the condition that develop 40micro of polylactic acid-Cap solutions L uniformly, and a drop does not spread on this water surface. After checking that the solvent had evaporated, the cast of the 50micro of the same solutions L was carried out on the water surface, and the honeycomb film was obtained by spraying high humidity air with an air pump. The honeycomb film which has floated on the water surface was moved to the frame (diameter of 5mm), and it considered as the free-standing

honeycomb film.
[0032]
[Formula 1]



Ｃａｐの構造式

[0033] Example 2: The rat liver origin hepatocyte (hepatocyte) was cultivated on the culture free-standing honeycomb film of the rat liver origin hepatocyte on a free-standing honeycomb film. First, seeding of the hepatocyte was carried out to one side of the free-standing honeycomb film produced in the example 1, and adhesion on a film was checked. Next, it cultivated by carrying out seeding of the hepatocyte also to the field of the opposite side. Culture was performed using the Williams'E culture medium within the CO2 incubator (CO2 concentration =5%, temperature =37 degree C, relative humidity = 80%). Seeding of the hepatocyte was carried out to both sides of the free-standing film which installed on the frame the PLLA-amphiphilic macromolecule cast film which does not have pore as an example of a comparison, and it cultivated on the same conditions.
[0034] When the free-standing honeycomb film produced in the example 1 was used, the solidified gestalt which has the thickness whose hepatocyte is about 20 micrometers in each field of a film was taken, and the three-dimensions cell aggregation object of the shape of a layer similar to liver tissue was formed. A result is shown in drawing 2 . This cell aggregation object discovered high albumin composition ability. When the free-standing film which, on the other hand, installed the PLLA-amphiphilic macromolecule cast film without pore on the frame was used, hepatocyte took the gestalt with a thickness of about 5 micrometers flattened remarkably, and the albumin secretion ability was about 30% as compared with the former.
[0035] Example 3: The rat embryo heart origin cardiac muscle cell (cardiac muscle cell) was cultivated on the culture free-standing honeycomb film of the rat embryo heart origin cardiac muscle cell on a free-standing honeycomb film. First, seeding of the cardiac muscle cell was carried out to one side of the free-standing honeycomb film produced in the example 1, and adhesion on a film was checked. Next, it cultivated by carrying out seeding of the cardiac muscle cell also to the field of the opposite side. Culture was performed using F-10 culture medium within the CO2 incubator (CO2 concentration =5%, temperature =37 degree C, relative humidity = 80%). Seeding of the cardiac muscle cell was carried out to both sides of the free-standing film which installed on the frame the PLLA-amphiphilic macromolecule cast film which does not have pore as an example of a comparison, and it cultivated on the same conditions.
[0036] When the free-standing honeycomb film produced in the example 1 was used, the myocardium cell aggregation object formed in each side of a film synchronized the pulsation rhythm with the whole film, and it was carrying out pulsation. That is, the three-dimensions myocardium cell aggregation object which the cardiac muscle cell pasted up on each side of a film has pasted up through a vesicular structure was formed. When the free-standing film which, on the other hand, installed the PLLA-amphiphilic macromolecule cast film without pore on the frame was used, the cardiac muscle cell did not synchronize the pulsation rhythm.
[0037]
[Effect of the Invention] According to the approach of this invention, it is possible to form the cell aggregation object structure of living body resemblance out of a living body, and to guide the manifestation of the function further.

[Translation done.]

* NOTICES *

1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] Drawing 1 is drawing showing the format of extension of a honeycomb structure object.
[Drawing 2] Drawing 2 shows the fluorescence microscope photograph (b) of a three-dimensions organization formed when a hepatocyte is cultivated in both sides of the free-standing honeycomb structure object film of polylactic acid in the conceptual diagram (a) of three-dimensions culture of a cell which used the free-standing honeycomb structure object film of polylactic acid for culture-medium material, and a list.

[Translation done.]

* NOTICES *

1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

DRAWINGS

[Drawing 1]

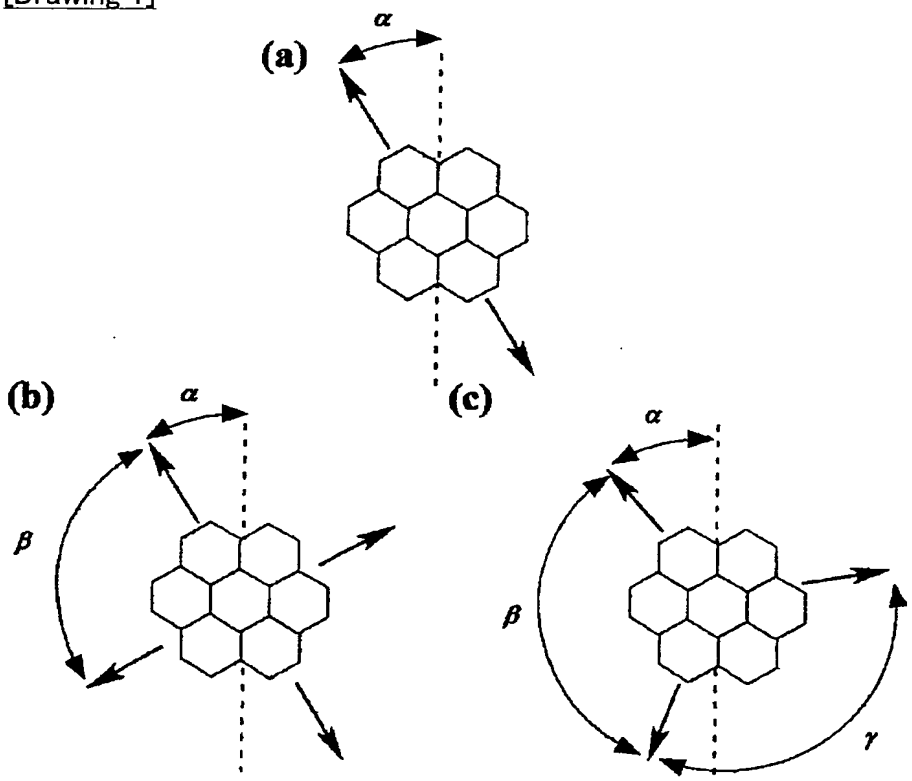


図1 ハニカム構造体の延伸の様式。(a)一軸延伸、(b)二軸延伸、(c)三軸延伸。
αは対称軸と延伸方向のなす角。β、γは延伸方向のなす角。

[Drawing 2]

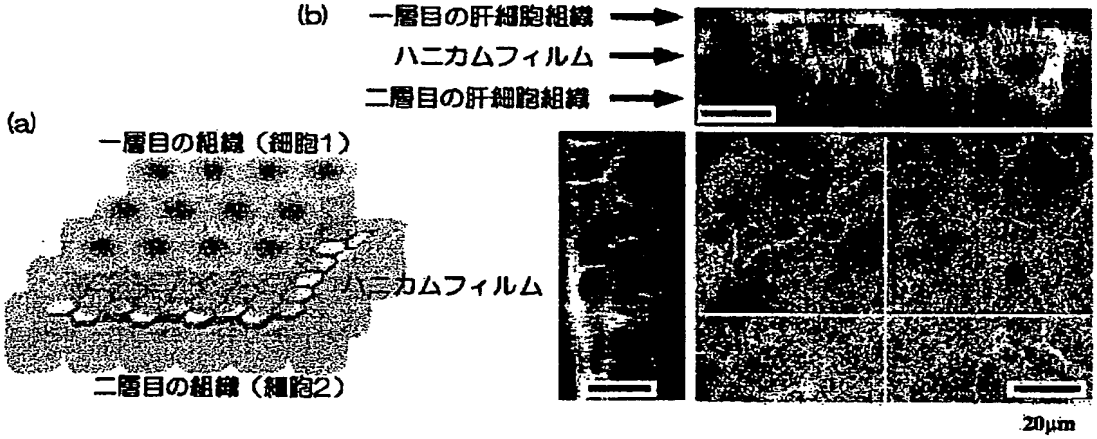


図 2 (a) ポリ乳酸の自己支持性ハニカム構造体フィルムを培養基材に用いた細胞の三次元培養
(b) 肝実質細胞をポリ乳酸の自己支持性ハニカム構造体フィルムの両面において培養した場合に形成される三次元組織の蛍光顕微鏡写真

[Translation done.]